PROPYLENE CARBONATE / t-BUTYL ALCOHOL HPV COMMITTEE
1250 Connecticut Avenue, N.W., Suite 700, Washington, DC 20036
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April 10, 2002

Ms. Christie Whitman, Administrator
US Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116
Via E-mail: oppt.ncic@epa.gov and chem.rtk@epa.gov

Re: Chemical Right-to-Know HPV Challenge Program Submission

Dear Ms. Whitman:

The Propylene Carbonate / t-Butyl Alcohol HPV Committee is pleased to submit the attached test plans and robust summaries for propylene carbonate (CAS RN 108-32-7) and t-butyl alcohol (CAS RN 75-65-0) under the HPV Challenge Program, AR-201. The Committee is submitting this information directly to directly to EPA with the understanding that a 120-day review period for public comment will follow this submission. The test plan and robust summaries for each chemical are being submitted in electronic format as PDF files.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Robert J. Fensterheim
Executive Director

AR201-13687A

Test Plan
for
Tertiary Butanol
CAS Number 75-65-0

USEPA HPV Challenge Program Submission

April 10, 2002

Submitted by:

Propylene Carbonate / t-Butyl Alcohol HPV Committee

Members:

Lyondell Chemical Company
Huntsman Corporation

Prepared by:
ToxWorks
1153 Roadstown Road
Bridgeton, New Jersey 08302-6640
Phone: 856-453-3478

I. Introduction

The Propylene Carbonate / t-Butyl Alcohol HPV Committee and its member companies have committed voluntarily to develop screening level human health effects, environmental fate and effects, and physicochemical test data for tertiary butyl alcohol under the Environmental Protection Agency's High Production Volume Challenge Program.

t-Butanol is manufactured in a closed system as part of the process of manufacture of propylene oxide. It is used primarily in the manufacture of methyl t-butyl ether, a gasoline oxygenate. During its manufacture and primary use, there is little human exposure to t-butanol. High purity t-butanol is used as a solvent. There are no known consumer markets for t-butanol.

Data Summary

	Data Available	Data Adequate	Testing Recommended
Melting point	Yes	Yes	No
Boiling point	Yes	Yes	No
Vapor Pressure	Yes	Yes	No
Partition Coefficient	Yes	Yes	No
Water Solubility	Yes	Yes	No
Stability in Water	No	No	Yes
Transport	No	No	Yes
Photodegradation	No	Yes	No
Biodegradation	Yes	Yes	No
Acute Toxicity to Fish	Yes	No	Yes
Acute Toxicity to Invert.	Yes	Yes	No
Acute Toxicity to aq. plants	No	No	Yes
Acute Tox – oral	Yes	Yes	No
Acute Tox – dermal	Yes	Yes	No
Gene tox in vivo – MN	Yes	Yes	No
Gene tox – vitro – Ames	Yes	Yes	No
Repeat dose- oral (90 day)	Yes	Yes	No
Repeat dos-inhal (90 day)	Yes	Yes	No
Repeat dose-derm (2 year)	Yes	Yes	No
Reproductive toxicity	Limited	No	Yes
Developmental tox	Yes	Yes	No

II. Test Plan and Rationale

A. Physical Chemical Data

The physical /chemical data for t-butanol are found in standard reference works. The underlying data were not found, but additional testing is not justified. No data on the photodegradation of t-butanol are available. Because t-butyl alcohol does not absorb light in the region of 290-800 nm, photodegradation testing is not required by guideline (see EPA 835.2310). Data on the transport of t-butanol between environmental compartments are not adequate.

A comparison of various biodegradation study designs was performed by Gerike and Fischer (1979). The results of studies of t-butanol were reported from the following: Zahn Wellens, MITI, AFNOR Test DOC, Sturm Test, OCED Screening Test, Closed Bottle Test, and Coupled Units Test. The authors did not provide detailed information about the methodologies used for each test; however, the paper provides an overall assessment of the biodegradability of TBA. Further testing is not warranted.

Recommended testing:

Transport and Distribution between Environmental Compartments **(EQC Level I modeling)**

The US EPA has acknowledged that computer modeling techniques are an appropriate method for estimating chemical partitioning among environmental compartments. A widely used fugacity model is the Equilibrium Criterion Model (EQC; Mackay et al., 1996). EPA has indicated that it accepts Level I fugacity data as an estimate of chemical distribution values. In EQC level I, distribution is calculated as percent partitioned to 6 compartments within a unit world. Level I data are basic partitioning data that allow for comparisons between chemicals and indicate the compartment(s) to which a chemical is likely to partition.

B. ECOTOXICITY

A study of limited value on the acute toxicity of TBA to goldfish (*Carassius auratus*) was published in 1979 (Bridie et al., 1979). The study does not meet EPA or OECD study guidelines, and does not contain details of study conduct or results. Therefore, an acute toxicity study in fish is recommended **(OECD guideline 203 or EPA guideline 850.1075)**.

The government of Germany performed a study (Kühn et al., 1989) of the toxicity of a number of chemicals to Daphnia. While some study details are not reported, the study is generally sound. Therefore, no additional testing is recommended.

No studies of TBA toxicity to algae are available, an acute toxicity study in algae is recommended **(OECD guideline 201)**.

Recommended testing:

Acute fish toxicity **(OECD Guideline 203)**
Algal toxicity **(OECD Guideline 201)**

C. MAMMALIAN TOXICITY

Acute Toxicity

Numerous acute toxicity tests are available on t-butyl alcohol. Oral, dermal and inhalation tests all meet OECD and EPA test guidelines. T-Butanol has low acute toxicity. The oral LD_{50} is 2733 mg/kg; the dermal LD_{50} is >20000 mg/kg. By inhalation, the LC50 is >14,100 ppm from a 4 hour whole body exposure to t-butanol vapor; ataxia and dyspnea were seen immediately post exposure at 9700 or 14,100 ppm. No further testing is recommended.

Repeated dose Toxicity

NTP performed short term and chronic carcinogenesis studies in both mice and rats by administration in drinking water. In rats, t-butanol caused kidney toxicity at concentrations of 1.25 to 5 mg/l and increased kidney tumors in male rats at 5 mg/l (420 mg/kg/day). In mice, t-butanol caused thyroid toxicity at concentrations of 10 to 20 mg/l and marginally increased thyroid tumors in females at 20 mg/l (2110 mg/kg). No further testing is recommended.

Mutagenesis Studies

There are two Ames assays; both are negative. There are two mouse lymphoma assays; both are negative. There is an in vitro sister chromatid exchange assay that was positive without activation, but negative with activation. Blood taken from mice in the 90 day NTP study were analyzed for micronuclei; TBA did not induce an increase in MN. The mutagenicity battery is satisfactory; no further mutagenicity testing is recommended.

Developmental Toxicity/Teratogenicity

Results from two developmental toxicity studies in mice are available; neither of the studies is compliant with either OECD or EPA guidelines for developmental toxicity

testing. However, there is sufficient information to determine that t-butyl alcohol has limited ability to cause malformations. No further developmental toxicity testing is recommended.

Toxicity to Reproduction

No studies of the effect of t-butanol on reproductive function are available. No adverse effects were observed in sex organs in rats or mice in the subchronic or chronic studies of t-butanol conducted by NTP. However, several observations (i.e., decreased fetal body weights in teratology studies, altered postnatal development) suggest that further study of reproductive toxicity is warranted. An enhanced OECD 421 study is proposed and would investigate the effect of t-butanol on mating behavior, preimplantation, embryonic and fetal development, parturition, and postnatal survival and development until weaning.

Recommended testing:

An enhanced **OECD Guideline 421** study on t-butyl alcohol is recommended. Key features include:

exposure of F0 males 14 days premating for minimum of 72 days total,
exposure of F0 female from 14 days premating through day 20 gestation and lactation days 5-21,
no direct exposure of offspring
mating allowed for 14 days of one male to one female
special attention will be paid to thyroids in F0 and F1 animals
sperm analysis will not be performed because it has already been evaluated in 90-day studies.

Robust Summaries
for

t-Butanol

CAS Number 75-65-0

USEPA HPV Challenge Program Submission

April 10, 2002

Submitted by:

Propylene Carbonate / t-Butyl Alcohol HPV Committee

Members:

Lyondell Chemical Company
Huntsman Corporation

Prepared by:
ToxWorks
1153 Roadstown Road
Bridgeton, New Jersey 08302-6640
Phone: 856-453-3478

T-Butanol
High Production Volume
Robust Summaries of Existing Studies

Data Point	Method	Value
1) MELTING POINT	Not Stated	25°C
Reference: IPCS Environmental Health Criteria 65, 1987, pp. 67-92.		
2) BOILING POINT	Not Stated	81°C
Reference: IPCS Environmental Health Criteria 65, 1987, pp. 67-92.		
3) VAPOUR PRESSURE	Not Stated	31 mm Hg @25°C
Reference: IPCS Environmental Health Criteria 65, 1987, pp. 67-92.		
4) PARTITION COEFFICIENT	Not stated	Log Pow: 0.37 Temp: Not stated
Reference: IPCS Environmental Health Criteria 65, 1987, pp. 67-92.		
5) WATER SOLUBILITY	Not stated	soluble in water
Reference: IPCS Environmental Health Criteria 65, 1987, pp. 67-92.		
6) PHOTODEGRADATION	Not required	does not absorb in region of 290-800 nm
Reference: US EPA test Guideline 835.2310		

9) BIODEGRADATION

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed (include calculated as one of the possible methods): Zahn-Wellens; MITI; Sturm; OECD Screen; Closed bottle
Test Type (test type/aerobic/anaerobic): Aerobic
GLP (Y/N): Not reported
Year (study performed): Not reported
Contact time (units): Varied by test
Innoculum: Varied by test

RESULTS

Degradation % after time:
Results: Zahn-Wellens – 96% removed after 6 days
MITI - 0% removed after 14 days
Sturm – 32% removed, 0% CO2 evolved

OECD screen – 29% converted
Closed bottle – 0% BODT at 30 days

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Degree of biodegradation very dependent on method used.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2, Key study
Remarks field for Data Reliability: Limited details, but important because several methods are compared.

REFERENCES (Free Text)

Gerike, P. and Fischer, W.K., 1979. A correlation study of biodegradability determinations with various chemicals in various tests. Ecotoxicol. Environ. Safety 3: 159-173.

OTHER

Last changed (administrative field for updating): 3/12/02 by ToxWorks

ECOTOXICITY ELEMENTS

10) ACUTE TOXICITY TO FISH

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed (experimental/calculated): Not stated
Type (test type): Static
GLP (Y/N): No data
Year (study performed): Not stated; published 1979
Species/Strain/Supplier: *Carassius auratus* (goldfish), strain and supplier not stated
Analytical monitoring: Total carbon or gas chromatography
Exposure period (unit): 24 hours
Statistical methods:
Remarks field for Test Conditions:
- Test fish (Age/length/weight, loading, pretreatment): Length = 6.2±0.7 cm; Weight = 3.3 ±1.0 g
- Test conditions:
· Details of test (static, semi-static, flow-through): Static

- Dilution water source: Not stated
- Dilution water chemistry (hardness, alkalinity, pH, TOC, TSS, salinity): Not stated
- Stock and test solution and how they are prepared: Not stated
- Concentrations dosing rate, flow-through rate, in what medium: Not stated
- Vehicle/solvent and concentrations: Not stated
- Stability of the test chemical solutions: Not stated
- Exposure vessel type (e.g., size, headspace, sealed, aeration, lighting, # per treatment): Glass tank - 42 x 28 x 28 cm, open, not aerated due to volatility of TBA
- Number of replicates, fish per replicate: 1 replicate, 6 fish
- Water chemistry in test (D.O., pH) in the control and one concentration where effects were observed: Not stated
- Test temperature range - Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): 20±1° C

RESULTS

Nominal concentrations (as mg/L): Not stated
Measured concentrations (as mg/L): Not stated
Unit (results expressed in what unit): mg/l
Element value (e.g. LC50, LClo, LL50, or LL0 at 48, 72 and 96 hours, etc., based on measured or nominal concentrations): >5000 mg/l at 24 hours
Statistical results, as appropriate:

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Submitter concludes that insufficient details are available to adequately characterize acute toxicity of t-butanol to fish.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 3
Remarks field for Data Reliability: Not guideline, poor documentation

REFERENCES (Free Text)

Bridie, A.L., Wolff, C.J.M., and Winter, M., 1979. The acute toxicity of some petrochemicals to goldfish. Water Research 13: 623-626.

OTHER

Last changed (administrative field for updating): 3/12/02 by ToxWorks

11) TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

No studies of the effects of t-butanol on algae were found.

12) ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G., DAPHNIA)

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed (experimental/calculated): German Institute of Standardization, 1982 DIN 38412, Part II.
Test type: Acute daphnia
GLP (Y/N): Not stated
Year (study performed): Not stated
Analytical procedures: Not stated
Species/Strain: *Daphnia magna* in-house cultures
Test details (static, semi-static, dosing rate, flow-through rate, etc.): Static
Statistical methods: Not stated
Remarks field for Test Conditions:
- Test organisms: No details provided.
- Test conditions:
· Stock solutions preparation (vehicle, solvent, concentrations) and stability: No solvent; stock solution in water, diluted with test water.
· Test temperature range: 20±1°C
· Exposure vessel type (e.g., size, headspace, sealed, aeration, # per treatment): 50 ml beaker, open, 2 used per concentration
· Dilution water source: Not stated
· Dilution water chemistry (hardness, alkalinity, pH, TOC, TSS, salinity, Ca/Mg ratio, Na/K ratio): Acid capacity ($K_{s4.3}$) = 0.8 mmol per l; hardness 2.4 mmol per l; Ca:Mg = 4:1; Na:K = 10:1; initial pH = 8.0±0.2
· Lighting (quality, intensity and periodicity): Not stated
· Water chemistry in test (D.O., pH) in the control and at least one concentration where effects were observed: D.O. > 2 mg/l.
- Element (unit) basis (i.e. immobilization): Immobilization
- Test design (number of replicates, individuals per replicate, concentrations): 2 replicates/concentration; 10 individuals/replicate
- Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): Not stated
- Exposure period: 48 hours
- Analytical monitoring: Not stated

RESULTS

Nominal concentrations in mg/L: Not stated
Measured concentrations in mg/L: Not stated
Unit [results expressed in what unit]: mg/l

EC50, EL50, LC0, LL0, at 24, 48 hours: EC50= 5504 (4607-6577) mg/l
Statistical results, as appropriate

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: The submitter concludes that t-butanol is not toxic to invertebrates.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2, Key study
Remarks field for Data Reliability: Appears to meet most of requirements of EPA OPPTS 850.1010, but some details not reported

REFERENCES (Free Text)

Kuhn, R., Pattard, M., Pernak, K.-D., and Winter, A., 1989. Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to *Daphnia magna*. Wat. Res. 23: 495-499.

OTHER

Last changed (administrative field for updating): 11/21/01 by ToxWorks

HEALTH ELEMENTS

13) ACUTE TOXICITY

13.1) ACUTE ORAL TOXICITY

Overview

Three acute oral toxicity studies on t-butyl alcohol have been performed. The results of all three studies should be considered together because they all provide solid evidence of the clinical and pathologic endpoints observed following a single oral dose of t-butanol. The following table tabulates the results:

Species	**LD_{50} (mg/kg)**	**Results**	**Reference**
Rat	2733	Ataxia, prostration, Bradypnea	IRDC, 1981
Rat	3500	Ataxia	Schaffarzick and Brown, 1952
Rabbit	3558	Dyspnea, Bradycardia, corneal reflexes	Munch, 1972

13.1.1) ACUTE ORAL TOXICITY IN RATS

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: EPA
Type: Acute oral
GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain: Rat, Sprague Dawley; Charles River, Portage MI
Sex: Both
No. of animals per dose: 5 per sex per dose
Vehicle: Not reported
Route of administration: Oral via stomach tube
Remarks field for Test Conditions:
- Age: Not reported
- Weight: 200 to 280 g
- Doses: 1500, 1950, 2535, 3296, and 4285 mg/kg
- Doses per time period: Presumed 1
- Volume administered or concentration: Not reported
- Post dose observation period: 14 days

RESULTS

Value [LD50 or LC50] with confidence limits if calculated: LD_{50} = 2733 ± 410 mg/kg;
Number of deaths at each dose level: 1500 = 0, 1950 = 3, 2535 = 5, 3296 = 5, and 4285 = 10.
Remarks field for Results: Ataxia, prostration, piloerection, bradypnea and hypoactivity were observed in the animals of all groups. Generally, surviving animals appeared normal by day 6. Body weights remained unchanged during the study. No compound related macroscopic lesions were noted.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1 – Key Study

REFERENCES (Free Text)

International Research and Development Corporation. 1981. Acute Oral Toxicity (LD_{50}) in Rats (EPA 8/78). Final Report 419-019, August 3, 1981

OTHER

Last changed: 11/21/01 by ToxWorks

13.1.2) ACUTE ORAL TOXICITY IN RATS

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: No details provided
Type: Acute oral
GLP (Y/N): No
Year (study performed): Not stated
Species/Strain: Rat, strain not stated
Sex: Not reported
No. of animals per dose: Not reported
Vehicle: Not reported
Route of administration: Oral, presumed gavage
Remarks field for Test Conditions:
- Age: Not reported
- Doses: Not reported
- Doses per time period: Not reported
- Volume administered or concentration: Not reported
- Post dose observation period: Not reported. At various times following dosing, the animals were tested for anticonvulsant activity.

RESULTS

Value [LD50 or LC50] with confidence limits if calculated: LD_{50} = 3500 mg/kg; ED_{50} (anticonvulsant activity) = 59 mg/kg; ED_{50} (ataxia) = 530 mg/kg.
Number of deaths at each dose level: Not reported
Remarks field for Results: No details reported

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2
Remarks field for Data Reliability: Lacking method details, not guideline study, but appears satisfactory. Not chosen as key study because better study available.

REFERENCES (Free Text)

Schaffarzick, R.W. and Brown, B.J. (1952) The Anticonvulsant Activity and Toxicity of Methyl-parafynol and Some other Alcohols. Science 116 663 – 665.

OTHER

Last changed: 11/21/01 by ToxWorks

13.1.3) ACUTE ORAL TOXICITY IN RABBITS

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: Limited details provided
Type: Acute oral
GLP (Y/N): No
Year (study performed): Not stated, published 1972
Species/Strain: Rabbit, strain not stated
Sex: Not reported
No. of animals per dose: Not reported
Vehicle: Dose of TBA followed by 5 ml saline
Route of administration: Oral via stomach tube
Remarks field for Test Conditions:
- Age: Not reported
- Weight: 1.5 to 2.5 kg
- Doses: Not reported
- Doses per time period: Not reported
- Volume administered or concentration: Administered neat; followed by 5 ml saline.
- Post dose observation period: Not reported. LD50 reported as deaths within 24 hours of dose.

RESULTS

Value [LD50 or LC50] with confidence limits if calculated: LD_{50} = 3558 mg/kg; Reported by author as : LD_{50} = 48 mMoles/kg
Narcotic Dose (general stupor and loss of voluntary movements in 50% of the animals): 19 mMoles/kg (1408mg/kg). Larger doses resulted in the observation of loss of corneal reflexes, nystagmus, dyspnea and bradycardia.
Number of deaths at each dose level: Not reported
Remarks field for Results: No details reported.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2
Remarks field for Data Reliability: Limited by lack of details, not guideline study. Not chosen as key study because better study available.

REFERENCES (Free Text)

Munch, J.C. (1972) Aliphatic Alcohols and Alkyl Esters: Narcotic and Lethal Potencies to Tadpoles and to Rabbits. Industrial Medicine 41: 31- 33.

OTHER

Last changed: 11/21/01 by ToxWorks

13.2) ACUTE DERMAL TOXICITY IN RABBITS

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: EPA
Type: Acute dermal
GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain: Rabbit, New Zealand White
Sex: Both
No. of animals per dose: 5 per sex per dose
Vehicle: None
Route of administration: Dermal, abraded skin
Remarks field for Test Conditions:
- Age: Not reported
- Weight: 2.5 to 3.2 kg
- Doses: 2000 mg/kg
- Doses per time period: 1
- Volume administered or concentration: Administered neat to abraded skin
- Post dose observation period: 14 days

RESULTS

Value [LD50 or LC50] with confidence limits if calculated: LD_{50} = > 2000 mg/kg
Number of deaths at each dose level: No deaths
Remarks field for Results: All animals exhibited erythema and fissuring of the skin, ranging from “very slight” to “moderate” and desquamation ranging from “very slight” to “slight”. Two males and one female reportedly exhibited significant weight loss during the study period (body weights not provided in report).
Gross pathological examination found thickening and encrustation at the treated site. These changes were considered incidental and not related to treatment. Hemorrhage was also observed at the treatment site in one male and one female. No other observations were noted.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

REFERENCES (Free Text)

International Research and Development Corporation, 1981. Acute Dermal Toxicity (LD50) Study in Rabbits (TSCA 7/79) (EPA 8/78) (OSHA). Final Report 419-020. July 28, 1981.

OTHER

Last changed: 3/11/02 by ToxWorks

13.3) ACUTE INHALATION TOXICITY IN RATS

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: EPA; exception: temperature and humidity not measured in chambers
Type: Acute inhalation
GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain: Rat, Charles River CD (Sprague-Dawley derived) Portage, MI
Sex: Both
No. of animals per dose: 5 per sex per dose
Vehicle: Air
Route of administration: Inhalation, vapor
Remarks field for Test Conditions:
- Age: Males: 52-56 days; females: 58-62 days
- Weight: Males: 257-287 g; females: 172-212 g
- Doses: 9700 and 14,100 ppm analyzed vapor concentration
- Doses per time period: 4 hours, whole body
- Post dose observation period: 14 days

RESULTS

Value [LD50 or LC50] with confidence limits if calculated: LC_{50} = > 14,100 ppm
Number of deaths at each dose level: No deaths at 9700 ppm, 3 of 10 deaths at 14,100 ppm
Remarks field for Results: Ataxia and prostration were observed during the exposure period at both exposure levels. Animals at the higher dose level also exhibited dyspsnea. During

the post exposure period, ataxia and dyspnea were observed in all animals of the lowest dose.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

REFERENCES (Free Text)

International Research and Development Corporation, 1981. LC50 Acute Inhalation Toxicity Evaluation in Rats. Final Report 419-020. August 19, 1981.

OTHER

Last changed: 11/21/01 by ToxWorks

GENETIC TOXICITY ELEMENTS

14) GENETIC TOXICITY IN VIVO (CHROMOSOMAL ABERRATIONS)

14.1) MICRONUCLEUS ASSAY IN MICE

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: NTP
Type (test type): Micronucleus
GLP (Y/N): No data
Year (study performed): 1996
Species: Mice
Strain: B6C3F1
Sex: Male
Route of administration: Intraperitoneal injection 3 days
Doses/concentration levels: 0, 312.5, 625, 1250 mg/kg/day
Exposure period: 3 daily injections
Statistical methods: Fisher's Exact and Trend test
Remarks field for Test Conditions:
- Age at study initiation: Not stated
- No. of animals per dose: 4
- Vehicle: Not stated
- Duration of test: 4 days: 3 days of exposure, analysis 24 hours later
- Frequency of treatment: Daily
- Sampling times and number of samples: 1 (24 hours)

- Control groups and treatment: Negative control and positive control (cyclophosphamide, CPA at 15 mg/kg)
- Clinical observations performed (clinical pathology, functional observations, etc.): Not stated
- Organs examined at necropsy (macroscopic and microscopic): Not stated
- Criteria for evaluating results (for example, cell types examined, number of cells counted in a mouse micronucleus test): 2000 cells counted, evaluation based on # MN/1000 polychromatic erythrocytes (PCE).
- Criteria for selection of M.T.D.: Not stated

RESULTS

Effect on mitotic index or PCE/NCE ratio by dose level by sex: MN/1000 PCE

Negative control	2.3±1.0
Positive control	14.2±1.6
t-butanol at 312.5 mg/kg/day	1.5±0.5
t-butanol at 625 mg/kg/day	1.4±0.1
t-butanol at 1250 mg/kg/day	1.7±0.3

Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal)
NOAEL(NOEL) (C)/LOAEL(LOEL) (C): Negative
Statistical results, as appropriate: Positive control statistically significant; all TBA groups not significant
Remarks field for Results:
- Mortality at each dose level by sex: None
- Description, severity, time of onset and duration of clinical signs at each dose level and sex: Not stated
- Body weight changes by dose and sex: Not stated
- Food/water consumption changes by dose and sex: Not stated

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Submitter concludes that t- butanol does not induce micronuclei.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2, Key study
Remarks field for Data Reliability: Results are from NTP database; they are not published or from official report.

REFERENCES (Free Text)

In-Vivo Cytogenesis Testing: Micronucleus Induction Results, NTP, From NIEHS Central Data Management, unpublished results, 1996.

OTHER

Last changed (administrative field for updating): 11/29/01

15) GENETIC TOXICITY IN VITRO (GENE MUTATIONS)

15.1) AMES ASSAY

15.1.1) AMES ASSAY – EG&G MASON STUDY

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: Ames
Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): Reverse mutation assay
System of testing [bacterial, non bacterial]: Bacterial
GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain or cell type and or cell line, bacterial or non-bacterial: *Salmonella typhimurium*, strains TA98, TA100, TA1535, TA1537, and TA1538
Metabolic activation: S-9 from male rat liver induced by Aroclor 1254.
Concentrations tested: 100, 500, 2500, 5000, 10,000 µg/plate
Statistical Methods: Doubling over background
Remarks field for Test Conditions:
- Test Design: Ames
· Number of replicates: 3
· Frequency of Dosing: Preincubation at 37°C for 20 minutes before plating for 48 hours
· Positive and negative control groups and treatment: Negative control and positive control for each strain: with activation: 2-aminoanthracene; without activation: TA98, TA 1538 – 2-nitroflurene; TA100, TA 1535 – 1,3-Propane Sultone; TA 1537 – 9-Amionacridine
- Solvent: None
- Description of follow up repeat study: None
- Criteria for evaluating results (e.g. cell evaluated per dose group): Doubling of revertants over negative control value.

RESULTS

Cytotoxic concentration
- With metabolic activation: >10,000 µg/plate
- Without metabolic activation: >10,000 µg/plate
Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal)

- With metabolic activation: Negative
- Without metabolic activation: Negative

Statistical results, as appropriate

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Author concluded that t-butanol did not induce mutations in Ames assay.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study
Remarks field for Data Reliability: High Quality; best documented with full study details.

REFERENCES (Free Text)

EG&G Mason Research Institute, 1981. Salmonella/Mammalian-Microsome Preincubation Mutagenicity Assay. T-Butyl Alcohol. Final Report 052-398-635-2, EG&G Mason, May 8, 1981.

OTHER

Last changed (administrative field for updating): 3/11/02 by ToxWorks

15.1.2) AMES ASSAY – NTP STUDY

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: NTP Procedure
Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): Reverse mutation assay
System of testing [bacterial, non bacterial]: Bacterial
GLP (Y/N): Not stated
Year (study performed): Not stated
Species/Strain or cell type and or cell line, bacterial or non-bacterial: *Salmonella typhimurium*, strains TA98, TA100, TA1535, and TA1537
Metabolic activation: S-9 from male rat and male hamster liver induced by Aroclor 1254.
Concentrations tested: 100, 333, 1000, 3333, 10,000 µg/plate
Statistical Methods: Not stated
Remarks field for Test Conditions:
- Test Design: Ames

· Number of replicates: 3
· Frequency of Dosing: Preincubation at 37°C for 20 minutes before plating for 48 hours
· Positive and negative control groups and treatment: Negative control and positive control for each strain: positive controls not explicitly stated
- Solvent: None
- Description of follow up repeat study: None
- Criteria for evaluating results (e.g. cell evaluated per dose group): Dose-related increase in revertants in any strain

RESULTS

Cytotoxic concentration:
- With metabolic activation: >10,000 µg/plate
- Without metabolic activation: >10,000 µg/plate
Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):
- With metabolic activation: Negative
- Without metabolic activation: Negative
Statistical results, as appropriate

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Submitter concludes the study is negative

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study;
Remarks field for Data Reliability: High Quality; equally good quality and same results as previous study.

REFERENCES (Free Text)

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., and Speck, W. 1987. Salmonella mutagenicity tests. III. Results from testing of 225 chemicals. Environ. Mutagen. 9(Suppl. 9): 1-109.

OTHER

Last changed (administrative field for updating): 3/11/02 ToxWorks

15.2) MOUSE LYMPHOMA ASSAY

15.2.1) MOUSE LYMPHOMA ASSAY – EG&G MASON STUDY

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: Mouse Lymphoma
Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): Forward mutation assay
System of testing [bacterial, non bacterial]: Mammalian cells
GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain or cell type and or cell line, bacterial or non-bacterial: L5178Y mouse lymphoma cells
Metabolic activation: S-9 from male rat liver induced by Aroclor 1254.
- Species and cell type: Mouse, L5178Y TK+/- lymphoma cells Clone 3.7.2C
- Quantity: 600,000 cells/ ml
- Induced or not induced: Both
- Concentrations tested: 1.7 to 32 µl/ml
- Statistical Methods: Doubling over controls
Remarks field for Test Conditions:
- Test Design
· Number of replicates: 2; also 2 studies
· Frequency of Dosing: Once
· Positive and negative control groups and treatment: Negative control and positive control: 0.5 and 1.0 µl/ml ethylmethanesulonate without activation and 5 and 7.5 µl/ml 7,12-dimethylbenzanthacene with activation.
- Solvent: Ethanol
- Description of follow up repeat study: None
- Criteria for evaluating results (e.g. cell evaluated per dose group): Doubling of mutant frequency over control

RESULTS

Cytotoxic concentration:
- With metabolic activation: 100 µl/ml
- Without metabolic activation: 100 µl/ml
Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal)
- With metabolic activation: - Negative
- Without metabolic activation: Negative
Statistical results, as appropriate

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Author concludes that t-butanol does not induce mutations in mouse lymphoma cells.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

REFERENCES (Free Text)

EG&G Mason Research Institute, 1981. Evaluation of test articles t-Butyl Alcohol – 99.9% (MRI #635) & t-Butyl Alcohol – Arconol (MRI # 636) for Mutagenic Potential Employing the L5178Y TK+/- Mutagenesis Assay. EG&G Mason Report 052-398-635-7 and 052-399-636-7, August 6, 1981.

OTHER

Last changed (administrative field for updating): 11/29/01 by ToxWorks

15.2.2) MOUSE LYMPHOMA ASSAY – NTP STUDY

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: NTP Methods
Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): Forward mutation assay
System of testing [bacterial, non bacterial]: Mammalian cells
GLP (Y/N): Not stated
Year (study performed): Not stated
Species/Strain or cell type and or cell line, bacterial or non-bacterial: L5178Y mouse lymphoma cells
Metabolic activation: S-9 from male rat liver induced by Aroclor 1254.
- Species and cell type: Mouse, L5178Y TK+/- lymphoma cells Clone 3.7.2C
- Quantity: 600,000 cells/ ml
- Induced or not induced: Both
Concentrations tested: 1.7 to 32 µl/ml
Statistical Methods: Pairwise comparison and trend test
Remarks field for Test Conditions:
- Test Design:
· Number of replicates: 2; study conducted twice
· Frequency of Dosing: Once
· Positive and negative control groups and treatment: Negative control and positive control: 15 µg/ml methylmethanesulonate without activation and 2.5 µl/ml MCA
- Solvent: Ethanol

- Description of follow up repeat study: None
- Criteria for evaluating results (e.g. cell evaluated per dose group): Significant increase in 1 of top 3 doses and significant trend

RESULTS

Cytotoxic concentration:
- With metabolic activation: >5000 µg/ml
- Without metabolic activation: >5000 µg/ml

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal)
- With metabolic activation: - Negative
- Without metabolic activation: - Negative

Statistical results, as appropriate: Positive control positive, no test groups positive

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Author concluded that t-butanol does not induce mutations in mouse lymphoma cells.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

REFERENCES (Free Text)

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., and Caspary, W.J., 1988. Responses of the L5178 tk+/tk- mouse lymphoma cell forward mutation assay to coded chemicals. II. 18 coded chemicals. Environ, Molec. Mutagen. 11: 91-118.

OTHER

Last changed (administrative field for updating): 11/29/01 by ToxWorks

15.3) IN VITRO DNA STUDIES – SISTER CHROMATID EXCHANGE

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: SCE
Type (e.g. reverse mutation assay, gene mutation study, cytogenesis assay, mammalian cell gene mutation assay, cytogenesis assay, etc.): DNA assay
System of testing [bacterial, non bacterial]: CHO cells

GLP (Y/N): Yes
Year (study performed): 1981
Species/Strain or cell type and or cell line, bacterial or non-bacterial: Chinese hamster ovary cell line
Metabolic activation: S-9 from male rat liver induced by Aroclor 1254.
- Species and cell type: Chinese hamster, ovary cells
- Quantity: 25
- Induced or not induced
Concentrations tested: 0.625 to 20 µl/ml
Statistical Methods: t-test
Remarks field for Test Conditions:
- Test Design
· Number of replicates: 2
· Frequency of Dosing: Once for 2 hours
· Positive and negative control groups and treatment: Negative control, solvent control, positive controls: triethylenemelamine without activation; cyclophosphamide with activation
· Number of metaphases analyzed: 50 cells
- Solvent: Ethanol
- Description of follow up repeat study: None
- Criteria for evaluating results (e.g. cell evaluated per dose group): Student's t-test with dose-response; doubling over negative/solvent control.

RESULTS

Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal)
- With metabolic activation: Significant increase at 5, 10, 20 µl/ml without dose-response; no doubling – author concluded it was negative
- Without metabolic activation: significant increase with dose-response at 5, 10 and 20 µl/ml; no doubling – author concluded it was positive
Statistical results, as appropriate

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. Authors concluded that t-butanol induced chromosomal aberrations *in vitro* in the presence of metabolic activation, but not in its absence.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

REFERENCES (Free Text)

EG&G Mason Research Institute, 1981. An In Vitro Evaluation of t-Butyl Alcohol-ARCONOL, Batch # A209411 to Produce Sister Chromatid Exchanges in Chinese Hamster

Ovary Cells. EG&G Mason Final Report 052-399-636-16, June 5, 1981, amended January 30, 1985.

OTHER

Last changed (administrative field for updating): 3/11/02 by ToxWorks

16) REPEATED DOSE TOXICITY

16.1) REPEATED DOSE TOXICITY – CHRONIC STUDY IN RATS

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: NTP standard study
Test type: Chronic study
GLP (Y/N): Yes
Year (study performed): 1986-1988
Species: Rat
Strain: F344/N
Route of administration, oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral, drinking water
Duration of test: 103 weeks
Doses/concentration levels: 0, 1.25, 2.5, and 5 mg/ml in males and 0, 2.5, 5, and 10 mg/ml in females
Sex: Both
Exposure period: Continuous 103 weeks
Frequency of treatment: Continuous
Control group and treatment: Yes, same as treated except no TBA in drinking water
Post exposure observation period: No
Statistical methods: Standard
Remarks field for Test Conditions:
- Test Subjects:
· Age at study initiation: 7 weeks
· No. of animals per sex per dose - Study Design: 60 males and 60 females
· Vehicle: Deionized water
· Satellite groups and reasons they were added: None
· Clinical observations performed and frequency (clinical pathology, functional observations, etc.): Twice daily, physical examination weekly, blood samples at 15 months for hematology, urinalysis month 15.
· Organs examined at necropsy (macroscopic and microscopic): Complete set of tissues evaluated for all animals.

RESULTS

NOAEL (NOEL): Not established
LOAEL (LOEL): 1.25 mg/ml in males; 2.5 mg/ml in females
Actual dose received by dose level by sex, if known: 0, 90, 200, and 420 mg/kg in males; 0, 180, 330, and 650 mg/ml in females.
Toxic response/effects by dose level: At 10 mg/ml – females – decreased survival, reduced body weight, nephropathy and mineralization, renal tubule hyperplasia, transitional epithelium hyperplasia in bladder, increased kidney weights.
At 5 mg/ml - males – decreased survival, reduced body weight, mineralization in kidney, transitional epithelium hyperplasia in bladder, increased renal tubule adenoma; females – increased kidney weights, nephropathy
At 2.5 mg/ml – males – decreased survival, mineralization in kidney, transitional epithelium hyperplasia; females – increased kidney weights, nephropathy
At 1.25 mg/ml – males – reduced body weight after week 65.
Statistical results, as appropriate
Remarks field for Results:
- Body weight (week 101): 454, 387, 374, 344, for males at 0, 1.25, 2.5, and 5 mg/ml; 331, 324, 316, 261, for females at 0, 2.5, 5, and 10 mg/ml
- Hematological findings incidence and severity: No treatment-related effects
- Mortality and time to death : Survival at 101 week: 12, 10, 4, 2 in males; 29, 28, 26, 17 in females
- Gross pathology incidence and severity: No treatment-related effects
- Organ weight changes: See above
- Histopathology incidence and severity: Kidney tumors (adenomas/carcinomas combined, based on combined standard and step sections: 8, 13, 19*, 13 for 0. 1.25, 2.5, and 5 mg/ml in males. None in females.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Author concluded there was "some evidence of carcinogenicity in males and no evidence of carcinogenicity in females". Submitter notes that Dr. Gordon Hard reviewed this study and concluded that t-butanol exacerbated chronic progressive nephropathy in male rats and also meet the criteria for alpha -2u-globulin mechanism and that these tumors are not relevant for human risk assessment. (Hard, G. 2001. Expert Evaluation of Renal Effects of tert-Butyl Alcohol in Rats and Mice. Report to Lyondell Chemical Company, Houston Texas, pp. 1-12.)

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

REFERENCES (Free Text)

National Toxicology Program (NTP), 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR-436 (NIH Publication No. 95-3167), pp. 1-313.

Cirvello, J.D., Radovsky, A., Heath, J.E., Farnell, D.R., and Lindamood, III, C., 1995. Toxicity and carcinogenicity of t-butyl alcohol in rats and mice following chronic exposure in drinking water. Toxicol. Ind. Health 11: 151-165.

OTHER

Last changed (administrative field for updating): 3/1202 by ToxWorks

16.2) REPEATED DOSE TOXICITY – CHRONIC STUDY IN MICE

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: NTP standard study
Test type: 2-year
GLP (Y/N): Yes
Year (study performed): 1986-1988
Species: Mouse
Strain: B6C3F1
Route of administration, oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral, drinking water
Duration of test: 103 weeks
Doses/concentration levels: 0, 5, 10, and 20 mg/ml
Sex: Both
Exposure period: Continuous 103 weeks
Frequency of treatment: Continuous
Control group and treatment: Yes, same as treated except no TBA in drinking water
Post exposure observation period: No
Statistical methods: Standard
Remarks field for Test Conditions:
- Test Subjects:
· Age at study initiation: 7 weeks
· No. of animals per sex per dose - Study Design: 60 males and 60 females
· Vehicle: Deionized water
· Satellite groups and reasons they were added: None
· Clinical observations performed and frequency (clinical pathology, functional observations, etc.): Twice daily, physical examination weekly,
· Organs examined at necropsy (macroscopic and microscopic): Complete set of tissues evaluated for mice.

RESULTS

NOAEL (NOEL): 5 mg/ml
LOAEL (LOEL): 10 mg/ml
Actual dose received by dose level by sex, if known: 0, 540, 1040, and 2070 mg/kg in males; 0, 510, 1020, and 2110 mg/kg in females.
Toxic response/effects by dose level: At 20 mg/ml – males – reduced survival, reduced body weight, increased thyroid follicular cell hyperplasia; females – reduced body weight, increased follicular cell hyperplasia, increased thyroid follicular cell adenoma
At 10 mg/ml – males – increased follicular cell hyperplasia, marginally increased follicular cell adenoma; females – increased follicular cell hyperplasia
At 5 mg/ml - males – No effect; females – No effect
Statistical results, as appropriate
Remarks field for Results:
- Body weight: 53, 53, 51, 50*, for males at 0, 5, 10, and 20 mg/ml at week 81, difference diminished after this; 60, 58, 57, 52*, for females at 0, 5, 10, and 20 mg/ml at week 81. *statistically significant
- Food/water consumption: No effect
- Description, severity, time of onset and duration of clinical signs: None
- Ophthalmologic findings incidence and severity: Not examined
- Hematological findings incidence and severity: Not examined
- Clinical biochemistry findings incidence and severity: Not examined
- Mortality and time to death: Survival at 101 week: 34, 44, 41, 23 in males; 43, 43, 46, 46 in females
- Gross pathology incidence and severity: No treatment-related effects
- Organ weight changes: See above
- Histopathology incidence and severity: Increased chronic inflammation of urinary bladder at 20 mg/ml in males and females. Increased follicular cell adenoma in females at 20 mg/ml (2/58, 3/60, 2/59, 9/59). Marginally increased follicular cell adenoma in males (1/60, 0/59, 4/59, 2/57);1 carcinoma seen at 20 mg/ml. Increased follicular cell hyperplasia males and females at 10 and 20 mg/ml

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Author concluded there was equivocal evidence of carcinogenicity in males and some evidence of carcinogenicity in females.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1, Key study

REFERENCES (Free Text)

National Toxicology Program (NTP), 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR-436 (NIH Publication No. 95-3167), pp. 1-313.

Cirvello, J.D., Radovsky, A., Heath, J.E., Farnell, D.R., and Lindamood, III, C., 1995. Toxicity and carcinogenicity of t-butyl alcohol in rats and mice following chronic exposure in drinking water. Toxicol. Ind. Health 11: 151-165.

OTHER

Last changed (administrative field for updating): 11/29/01 by ToxWorks

16.3) REPEATED DOSE TOXICITY – SUBCHRONIC STUDY IN RATS

TEST SUBSTANCE

Identity: t-Butyl alcohol

METHOD

Method/guideline followed: NTP standard study
Test type: 13 weeks
GLP (Y/N): Yes
Year (study performed): 1985
Species: Rat
Strain: F344/N
Route of administration, oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral, drinking water
Duration of test: 92-94 days
Doses/concentration levels: 0, 2.5, 5, 10, 20 and 40 mg/ml
Sex: Both
Exposure period: Continuous 92-94 days
Frequency of treatment: Continuous
Control group and treatment: Yes, same as treated except no TBA in drinking water
Post exposure observation period: No
Statistical methods: Standard
Remarks field for Test Conditions:
- Test Subjects
· Age at study initiation: 6 weeks
· No. of animals per sex per dose - Study Design: 10 males and 10 females
· Vehicle: Deionized water
· Satellite groups and reasons they were added: None
· Clinical observations performed and frequency (clinical pathology, functional observations, etc.): Twice daily, physical examination weekly, blood samples at 2 and 13 weeks for hematology and clinical chemistry, urinalysis weeks 2 and 13.

· Organs examined at necropsy (macroscopic and microscopic): Complete set of tissues evaluated for rats in 0, 20, and 40 mg/ml dose groups; target organs in lower dose groups.

RESULTS

NOAEL (NOEL): 2.5 mg/ml in males, except for kidney for which a NOAEL was not established; 5 mg/ml in females

LOAEL (LOEL): 5 mg/ml in males; 10 mg/ml in females

Actual dose received by dose level by sex, if known: 0, 230, 490, 840, 1520, and 3610 mg/kg in males; 0, 290, 590, 850, 1560, and 3620 mg/ml in females.

Toxic response/effects by dose level: At 40 mg/ml – death of all males and 6 of 10 females, nephropathy, transitional epithelial hyperplasia in urinary bladder, increased liver and kidney weights.

At 20 mg/ml – males – reduced body weight, nephropathy, hyaline droplet accumulation, increased liver and kidney weights; females – nephropathy, increased liver and kidney weights.

At 10 mg/ml – males – reduced body weight, nephropathy, hyaline droplet accumulation, increased liver and kidney weights; females – nephropathy, increased liver and kidney weights.

At 5 mg/ml - males – reduced body weight, nephropathy, hyaline droplet accumulation, increased kidney weights; females –increased liver and kidney weights.

At 2.5 mg/ml – males - nephropathy, hyaline droplet accumulation, increased kidney weights; females –increased liver and kidney weights.

Statistical results, as appropriate

Remarks field for Results:

- Body weight: 355, 341, 339, 313, 294 g for males at 0, 2.5, 5, 10, and 20 mg/ml; 179, 183, 180, 181, 176, 141 g for females at 0, 2.5, 5, 10, 20, and 40 mg/ml
- Food/water consumption:
- Description, severity, time of onset and duration of clinical signs: No effects
- Ophthalmologic findings incidence and severity: Not examined
- Hematological findings incidence and severity: Decreased RBC count and hemoglobin in males at 10 and 20 mg/ml; no differences in females
- Clinical biochemistry findings incidence and severity: Increased sorbitol dehydrogenase in males at 10 and 20 mg/ml; increase alanine aminotransferase in females at 20 and 40 mg/ml.
- Mortality and time to death: 10 /10 males at 40 mg/ml weeks 4, 5, 5, 6, 7, 8, 8, 8, 12, 12; 6 / 10 females at 40 mg/ml weeks 2, 8, 8 10 ,12, 12
- Gross pathology incidence and severity: Calculi in urinary bladder in males at 40 mg/ml
- Organ weight changes: See above
- Histopathology incidence and severity: See above

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Author concluded 5 mg/ml in males and 10 mg/ml in females were maximum tolerated doses for 2 year study. Submitter notes That Dr. Michael McClain reviewed the

thyroid effects in mice. He concluded that the increased thyroid tumors are compatible with a proliferative response seconday to hormone imbalance caused by microsomal enzyme induction. (McClain, R.M. 2001. Assessment of the Thyroid Follicular Cell Tumor Findings from Toxicity and Carcinogenicity Studies with tert-Butyl Alcohol in B6C3F1 Mice, Report to Lyondell Chemical Company, Houston, Texas, pp. 1-14.)

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1
Remarks field for Data Reliability: Not regarded as key study because chronic study available.

REFERENCES (Free Text)

National Toxicology Program (NTP), 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR-436 (NIH Publication No. 95-3167), pp. 1-313.

Lindamood *et al.*, 1992. Subchronic Toxicity Studies of t-butyl alcohol in Rats and Mice. Fund. Appl. Toxicol. 19: 91-100.

OTHER

Last changed (administrative field for updating): 3/12/02 by ToxWorks

16.4) REPEATED DOSE TOXICITY – SUBCHRONIC STUDY IN MICE

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: NTP standard study
Test type: 13 weeks
GLP (Y/N): Yes
Year (study performed): 1985
Species: Mouse
Strain: B6C3F1
Route of administration, oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral, drinking water
Duration of test: 94-95 days
Doses/concentration levels: 0, 2.5, 5, 10, 20 and 40 mg/ml
Sex: Both
Exposure period: Continuous 94-95 days
Frequency of treatment: Continuous

Control group and treatment: Yes, same as treated except no TBA in drinking water
Post exposure observation period: No
Statistical methods: Standard
Remarks field for Test Conditions:
- Test Subjects:
· Age at study initiation: 6 weeks
· No. of animals per sex per dose - Study Design: 10 males and 10 females
· Vehicle: Deionized water
· Satellite groups and reasons they were added: None
· Clinical observations performed and frequency (clinical pathology, functional observations, etc.): Twice daily, physical examination weekly, blood samples at 2 and 13 weeks for hematology.
· Organs examined at necropsy (macroscopic and microscopic): Complete set of tissues evaluated for mice in 0, 20, and 40 mg/ml dose groups; target organs in lower dose groups.

RESULTS

NOAEL (NOEL): 10 mg/ml in males; 10 mg/ml in females
LOAEL (LOEL): 20 mg/ml in males; 20 mg/ml in females
Actual dose received by dose level by sex, if known: 0, 350, 640, 1590, 3940, and 8210 mg/kg in males; 0, 500, 820, 1660, 6430, and 11,620 mg/kg in females.
Toxic response/effects by dose level: At 40 mg/ml – males - death of 6 of 10, reduced body weight, transitional epithelial hyperplasia in urinary bladder; females – death of 4 of 10, reduced body weight, increased liver and kidney weights, transitional epithelial hyperplasia in urinary bladder.
At 20 mg/ml – males – reduced body weight, transitional epithelial hyperplasia in urinary bladder; females – death of 1 of 10.
At 10 mg/ml – males - No effect; females – No effect.
At 5 mg/ml – males – No effect; females – No effect.
At 2.5 mg/ml – males - No effect; females – No effect.
Statistical results, as appropriate
Remarks field for Results:
- Body weight: 38, 38, 38, 37, 33, and 29 g for males at 0, 2.5, 5, 10, 20, and 40 mg/ml; 30, 31, 29, 31, 28, and 25 g for females at 0, 2.5, 5, 10, 20, and 40 mg/ml
- Food/water consumption:
- Description, severity, time of onset and duration of clinical signs: No treatment-related effects other than related to deaths
- Ophthalmologic findings incidence and severity: Not examined
- Hematological findings incidence and severity: Not examined
- Clinical biochemistry findings incidence and severity: Not examined
- Mortality and time to death: 6 of 10 males at 40 mg/ml weeks 4, 5, 7, 7, 9, 13; 4 of 10 females at 40 mg/ml weeks 2, 5, 9, 9; 1 of 10 females at 20 mg/ml week 10
- Gross pathology incidence and severity: Increased thickness of urinary bladder in males at 20 and 40 mg/ml
- Organ weight changes: Increased liver and kidney weights in females at 40 mg/ml

- Histopathology incidence and severity: Hyperplasia of the urinary bladder transitional epithelium

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Author concluded 20 mg/ml in males and females were maximum tolerated doses for 2 year study.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 1
Remarks field for Data Reliability: Not regarded as key study because chronic study is available.

REFERENCES (Free Text)

National Toxicology Program (NTP), 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR-436 (NIH Publication No. 95-3167), pp. 1-313.

Lindamood *et al.* , 1992. Subchronic Toxicity Studies of t-butyl alcohol in Rats and Mice. Fund. Appl. Toxicol. 19: 91-100.

OTHER

Last changed (administrative field for updating): 11/29/01 by ToxWorks

17) TOXICITY TO REPRODUCTION

TEST SUBSTANCE

Identity: t-Butanol

METHOD

Method/guideline followed: None
Type (one generation, two generation, etc.): Evaluation of sex organs in subchronic study
GLP (Y/N): Yes
Year (study performed): 1986
Species: Rat and Mouse
Strain: F344/N and B6C3F1
Route of administration - oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral, drinking water
Doses/concentration levels: 0, 2.5, 5, 10, 20 and 40 mg/ml
Sex: Both

Control group and treatment: Yes, same as treated except no TBA in drinking water
Frequency of treatment: Continuous
Duration of test: 92-96 days
Statistical methods

RESULTS

NOAEL (NOEL) and LOAEL (LOEL): 20 mg/ml in females; 40 mg/ml in males
Actual dose received by dose level by sex if known: rats: 0, 230, 490, 840, 1520, and 3610 mg/kg in males; 0, 290, 590, 850, 1560, and 3620 mg/ml in females; mice: 0, 350, 640, 1590, 3940, and 8210 mg/kg in males; 0, 500, 820, 1660, 6430, and 11,620 mg/ml in females.
Statistical results, as appropriate
Remarks field for Results:
- Body weight: Rats: decreased in males at 5, 10, 20 mg/ml; females at 40 mg/ml; Mice: decreased in males at 20 and 40 mg/ml and in females at 40 mg/ml.
- Description, severity, time of onset and duration of clinical signs:
- Changes in estrus cycles: Rats: No effect on estrous cycle length or percentage of time spent in the various estrous stages. Mice: No effect on percentage of time spent in various stages of estrous, but increased estrous cycle length at 40 mg/ml (40% died, also reduced body weight).
- Effects on sperm: Rats and mice: No effect on sperm motility or sperm morphology.

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. The significance of the increased estrus cycle length observed in female mice receiving 11,620 mg/kg (40 mg/ml in water) t-butanol with a 40% rate of mortality is questionable. Altered estrus cycle length (typically increased) is common in female animals with altered homeostasis due to systemic toxicity. Of significance was the lack of altered estrus cycle length in female mice receiving 6430 mg/kg (20 mg/ml in water), with a 10% incidence of mortality.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2
Remarks field for Data Reliability: studies well done, but assess endpoints of reproductive organs, not reproduction

REFERENCES (Free Text)

National Toxicology Program (NTP), 1995. NTP Technical Report on the Toxicology and Carcinogenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR-436 (NIH Publication No. 95-3167), pp. 1-313.

OTHER

Last changed (administrative field for updating): 11/29/01 by ToxWorks

18) DEVELOPMENTAL TOXICITY/TERATOGENICITY

18.1) POSTNATAL DEVELOPMENTAL TOXICITY STUDY IN MICE

TEST SUBSTANCE

Identity: t-Butanol
Remarks field for Test Substance: No description of source or purity

METHOD

Method/guideline followed: Teratogenicity study with developmental landmarks
GLP (Y/N): No data
Year (study performed): Not stated, accepted for publication 1981
Species: Mice
Strain: Swiss Webster
Route of administration - oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other: Oral (in liquid diet)
Doses/concentration levels: 0.5, 0.75 and 1.0 % in diet.
Sex: Females
Exposure period: Days 6-20 of gestation
Frequency of treatment: Continuous
Control group and treatment: Negative control: Liquid diet without t-butanol; Positive control: 3.6% ethanol
Duration of test: Through postnatal day 22
Statistical methods: SAS package: t-test, linear regression
Remarks field for Test Conditions:
- Age at study initiation: 8 to 10 weeks old
- Number of animals per dose per sex: 15
- Vehicle: Modified Lieber and Decarli liquid diet
- Clinical observations performed and frequency: Not stated
- Mating procedures (M/F ratios per cage, length of cohabitation, proof of pregnancy): 1 male/3 females until mated; sperm plug
- Parameters assessed during study (maternal and fetal): Maternal: body weight, length of gestation
- Organs examined at necropsy (macroscopic and microscopic): Not stated

RESULTS

NOAEL (NOEL) and LOAEL (LOEL) maternal toxicity: Not stated
NOAEL (NOEL) and LOAEL (LOEL) developmental toxicity
Actual dose received by dose level by sex if available

Maternal data with dose level (with NOAEL value). Provide at a minimum qualitative descriptions of responses where dose related effects were seen.: Authors report "significant postnatal maternal nutritional and behavioral factors affecting lactation and/or nesting behavior were evident at the higher concentrations of alcohol" without description or quantization of those effects. Decreased body weight at 0.75 and 1.0 % t-butanol (TBA).

Fetal data with dose level (with NOAEL value). Provide at a minimum qualitative descriptions of responses where dose related effects were seen: Two-day delay in eye opening at 1% TBA, reduced pup weight at 1 % TBA, less obvious in pups that were cross fostered; effects were reported for cliff avoidance, righting reflex, open field activity, and roto-rod performance in pups from TBA exposed dams, but the results were not compared to control values, so a NOAEL was not reported.

Statistical results, as appropriate

Remarks for Results:

- Mortality and day of death: None
- Number pregnant per dose level: Not stated
- Number aborting: Not stated
- Number of resorptions, early/late if available: Not stated
- Number of implantations: Not stated
- Pre and post implantation loss, if available: Not stated
- Number of corpora lutea (recommended): Not stated
- Duration of Pregnancy: Not applicable; terminated on gestational day 20
- Body weight: At gestational day 20: Control - 43.3 g, 0.5% TBA – 44.3 g; 0.75% TBA – 41.0 g; 1.0% TBA – 39.0 g
- Food/water consumption: Pair-fed from 1% TBA consumption
- Description, severity, time of onset and duration of clinical signs: Not reported
- Fetal data, provide at a minimum qualitative descriptions of responses where dose related effects were seen:

· Litter size and weights:

	0	0.5%	0.75%	1.0%
Neonates/litter =	10.4±4.0	10.3±4.4	7.4±2.3	5.3±2.8
Fetal wt, day2 =	1.78±0.21	1.66±0.24	1.45±0.14	1.10±0.10

· Number viable (number alive and number dead): Total Number Stillborn: 3, 6, 14, 20 at 0, 0.5, 0.75 and 1.0% TBA
· Sex ratio: Not reported
· Postnatal growth (depending on protocol): at postnatal day 10 (estimated from graph): 6.9, 6.5, 6.0 and 4.0 g/pup for 0, 0.5, 0.75, and 1.0% TBA
· Postnatal survival (depending on protocol): Not reported
· Grossly visible abnormalities, external, soft tissue and skeletal abnormalities: Not reported

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter. The submitter concludes there were developmental delays in pups whose dams experienced significant maternal toxicity from exposure to 0.75 or 1.0% t-butanol in the liquid diet.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2
Remarks field for Data Reliability: Not guideline study, incomplete evaluation; not able to calculate actual dose (mg/kg) of t-butanol.

REFERENCES (Free Text)

Daniel, M.A. and Evans, M.A., 1982. Quantitative Comparison of Maternal Ethanol and Maternal Tertiary Butanol Diet on Postnatal Development. J. Pharm. Exp. Therap. 222: 294-300.

OTHER

Last changed (administrative field for updating): 11/21/01by ToxWorks

18.2) DEVELOPMENTAL TOXICITY/TERATOGENICITY IN MICE

TEST SUBSTANCE

Identity: t-Butanol
Remarks field for Test Substance: No description of test material in paper

METHOD

Method/guideline followed : Not specified
GLP (Y/N): No data
Year (study performed): Not specified
Species: Mouse
Strain: CBA/J and C57BL/6J
Route of administration - oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapor, gas, particulate), other : Gavage
Doses/concentration levels: 10.5 mmole/kg twice per day (1550 mg/kg/day)
Sex: Female
Exposure period: Days 6-18 of gestation
Frequency of treatment: Twice daily
Control group and treatment: Similar size group for each strain; received tap water by gavage twice daily
Duration of test: Through day 18 of gestation
Statistical methods: t-test, chi squared
Remarks field for Test Conditions:
- Age at study initiation: 25-30 weeks old
- Number of animals per dose per sex: CBA/J: control = 7 dams; TBA = 12 dams; C57BL/6J: control = 5 dams; TBA = 9 dams
- Vehicle: Tap water
- Clinical observations performed and frequency: Not stated

- Mating procedures (M/F ratios per cage, length of cohabitation, proof of pregnancy): Not stated
- Parameters assessed during study (maternal and fetal): Not stated
- Organs examined at necropsy (macroscopic and microscopic): Uterus, fetuses

RESULTS

NOAEL (NOEL) and LOAEL (LOEL) maternal toxicity: Not reported
NOAEL (NOEL) and LOAEL (LOEL) developmental toxicity: NOAEL not established
Actual dose received by dose level by sex if available: 1550 mg/kg/day
Remarks for Results:
- Mortality and day of death: Not stated
- Number pregnant per dose level: CBA/J: 7 and 12; C57BL/6J: 5 and 9
- Number aborting: Not reported
- Number of resorptions, early/late if available: mean/litter: CBA: 1.42±0.72 control; 3.09±0.57 TBA; C57Bl/6J: 0.80±0.57 control; 4.22±1.29 TBA
- Number of implantations: (Mean) – CBA: 9.4; 7.8; C57BL/6 8.8; 7.6
- Pre and post implantation loss, if available: Not reported
- Number of corpora lutea (recommended): Not reported
- Duration of Pregnancy: Terminated day 18
- Body weight: Not reported
- Food/water consumption: Not reported
- Description, severity, time of onset and duration of clinical signs: Not reported
- Organ weight changes, particularly effects on total uterine weight: Not reported
- Histopathology incidence and severity: Not reported
- Fetal data, provide at a minimum qualitative descriptions of responses where dose related effects were seen

	CBA		C57BL/6	
	TBA	control	TBA	control
· Litter size: Live fetuses: mean/litter	8.00±1.00	4.75±1.00	8.00±0.45	3.33±1.44
· Litter weights: mean/litter (g±sem)–	0.80±0.01	0.77±0.02	0.94±0.02	0.90±0.03
· Resorptions (mean ±sem):	1.42±0.72	3.09±0.57	0.80±0.49	4.22±1.29

· Sex ratio: Not reported
· Grossly visible abnormalities, external, soft tissue and skeletal abnormalities: No significant differences

CONCLUSIONS

Remarks field with the ability to identify source of comment, i.e. author and/or submitter: Authors concluded that TBA at 1550 mg/kg/day caused increased resorptions, but not developmental anomalies.

DATA QUALITY

Reliabilities (Klimisch Code, if used, possibly a flag if 'key study'): 2
Remarks field for Data Reliability: Not guideline study, incomplete evaluation.

REFERENCES (Free Text)

Faulkner, T.P., Wiechart, J.D., Hartman, D.M., and Hussain, A.S., 1989. The effects of prenatal tertiary butanol administration in CBA/J and C57BL/6J mice. Life Sci. 45: 1989-1995.

OTHER

Last changed (administrative field for updating): 3/11/02 by ToxWorks